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California Nevada Fish Health Center

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Delta Smelt Technical Report - Fish Health Services and Culture Support for Delta Smelt Held at Livingston Stone National Fish Hatchery

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Introduction

The delta smelt (*Hypomesus transpacificus*) is a pelagic fish that has become a major focus of concern in California. Natural populations have declined dramatically in recent years (80%) and protective measures at State and Federal pumping facilities have restricted agricultural water allocations (Center for Biological Diversity, 2006). Scientific knowledge about the species has increased, but many aspects of its biology are still unknown.

Delta smelt are endemic to the upper Sacramento-San Joaquin estuary and were listed as a threatened species in 1993 due to population decline (Moyle, 1992; Register, 1993; Service U. F., Oct 2007). Due to the low population abundance, the species might be in danger of extinction (Service U. F., Feb 2007). Factors thought to contribute to the declining population include reduction in freshwater outflow, competition and predation from invasive aquatic species, and reduction in the abundance of key food organisms (Service U. F., Delta Smelt Species Account, 2009; Register, 1993).

Delta smelt have an unusual life history because they live entirely within the confines of the estuary (Bennett, 2005). They have a small geographic range in the low salinity habitat associated with the freshwater saltwater mixing zone of the estuary (Bennett, 2005; Moyle, 1992; Service U. F., Oct 2007). The exception to this range is when adults move upstream to river channels to spawn (Moyle, 1992). Spawning occurs in freshwater during the winter and spring (February to May) at water temperatures between 7 and 15°C (Moyle, 1992). Larval and juvenile fish are transported by flow downstream from freshwater areas to low salinity areas near the upper end of the mixing zone. In the fall, adults begin their upstream migration to freshwater spawning areas (Bennett, 2005). Delta smelt are an annual species, completing their life cycle in one year however there is a small portion that survive two years (Bennett, 2005).

Due to low population abundance, the U.S. Fish and Wildlife Service established a genetic refugial population to propagate fish and maintain this important population of fish for future restoration activities. The current facilities that produce delta smelt are the University of California Davis (UCD) Fish Conservation & Culture Lab (FCCL) in Byron, CA and the FWS Livingston Stone National Fish Hatchery (LSNFH) in Shasta Lake, CA. The FCCL was established in 1993 and has developed a successful culture program for delta smelt (Service U. F., Oct 2007). The focus of the FCCL is to maintain the genetic composition of the population utilizing captured wild delta smelt, and conducting applied research on this species. LSNFH Delta smelt program was initiated in 2006 with the primary goals of investigating aquaculture methods and broodstock management for this species, and to serve as a back-up site for a refugial population. The goal of the refugial population breeding plan is to propagate delta smelt in a separate culture facility from the FCCL, to safeguard against potential catastrophic losses at either culture facility or in the wild population.

The California-Nevada Fish Health Center (Ca-Nv FHC) provides fish health support to FCCL and LSNFH including diagnostic and inspection services, as well as technical

information to support best culture practices and broodstock management. Cultured stocks of delta smelt have a history of predisposition to mycobacterial infections, thought to originate from wild population sources (Antonio D.B, 2000), and which can be exacerbated in intensive culture conditions. In 2005, mycobacterium was detected at the FCCL in fish suffering from lethargy and chronic mortality (Ca-Nv FHC unpublished data). Two years later, in 2007, delta smelt reared at LSNFH experienced an epizootic of mycobacteriosis in adults that had been held for reconditioning, an early culture effort to invoke a second spawn from adult females. At that time, cultures were collected and presumptively identified as mycobacteria by acid fast stain and growth characteristics at various temperatures. Mycobacterial cultures were sequenced and aligned by Chris Whipps of Oregon State University, and identified as a composition of multiple strains: *Mycobacteria marinum*, *M. fortuitum* and *M. chelonae* (a subspecies of the closely related *M. salmoniphilum*).

Mycobacterial infections can be latent in wild or cultured populations until stress induction causes a progression to a clinical disease state. The 2007 epizootic occurred in post spawning females held for re-conditioning, but many aspects of the culture environment can lead to disease outbreaks (handling, high densities, poor water quality, etc.). The attempts to recondition females was discontinued in 2007 and no further mycobacterial disease outbreaks have occurred in the past three years of production and brood stock operations. Additionally, Delta smelt mortalities are frozen back and subsampled for mycobacterial testing by Quantitative Polymerase Chain Reaction (QPCR) assays to closely monitor the bacterial prevalence in the cultured population, and minimize the potential for disease outbreaks.

Delta smelt are held in a quarantine/isolation facility at LSNFH. Biosecurity measures, and precautions for staff handling adult fish, are in place and include: UV disinfection of water supply and effluent, disinfection protocols and footbaths, isolation of production groups, dedicated equipment for individual tanks and groups, minimal handling of fish including water-to-water transfers, and incorporation of oxytetracycline prophylactic water baths during spawning or extended handling of fish.

In 2010, the Ca-Nv FHC provided technical support and monitoring of delta smelt which included a fish health inspection prior to transfer of sub-adult fish to Livingston Stone NFH, chemotherapeutic bioassays conducted on larval and adult fish to investigate treatment options if disease outbreaks should occur, and continued testing for mycobacterium in production mortalities. These efforts are important in order to: protect LSNFH and the delta smelt program from unintentional introduction of fish pathogens, to provide information regarding treatment options and efficacy, and to manage latent mycobacteria infections present in wild and cultured stocks of delta smelt. This report summarizes the fish health work performed in fiscal year 2010 (Oct 2009 – Sept 2010).

Fish Health Inspections and Monitoring

Transfer of Delta Smelt (*Hypomesus transpacificus*) for Refugial Population

In October 2009, the California-Nevada Fish Health Center conducted a fish health inspection (Case # 10-001) prior to transfer of mixed family group (MFG) sub-adults from FCCL to LSNFH. Sixty live fish were euthanized, necropsy was performed, and tissues were processed for external parasite screening and specific fish pathogen assays outlined in FWS Fish Health Policy. Additionally, the exam included screening 45 fish for sub-clinical mycobacterial infection by QPCR. The Fish Health Inspection Report is included in Appendix II. All samples were tested according to U.S. Fish and Wildlife Service Standard Procedures for Aquatic Animal Health Inspections (SPAHI). Laboratory methods used for the fish health inspections are described in Appendix 1.

Virology was conducted on 5-pool visceral tissue that was removed from each fish and assayed for virus. No evidence of viral cytopathic effects was observed during the tissue culture incubation period of 18d at 18°C.

Bacteriology was conducted on thirty fish and *Aeromonas* spp, *Pseudomonas* spp and *Micrococcus* spp were detected in 10% of the fish examined and not considered significant bacterial infections. Pathogenic bacteria, such as *Aeromonas salmonicida* and *Yersinia ruckeri* were not found. Fish were also screened for bacterial kidney disease using a fluorescent antibody test (FAT). Twenty five individual fish tested by FAT were all negative.

Delta smelt production lots were also tested for mycobacterial infection over the course of the rearing period. Ten 3-pool samples were screened for *Mycobacteria* spp. by QPCR. *Mycobacterium* spp was found in 60% of the samples indicating the presence of this bacterium in both the culture population and sub-adults reared at FCCL.

Biological Assays to Investigate Potential Drug Treatments

In January 2010, excess delta smelt (BY2009 from LSNFH and BY2008 from the FCCL) were used in a biological assay conducted to assess the tolerance of delta smelt to three commonly used chemotherapeutants. The three treatments of interest were salt (Mix-N-Fine Salt, Cargill Salt), Chloramine T (INAD # 9321, B.L. Mitchell, Inc.), and Kordon's Rid-Ich+ (Novalek, Inc.). These drugs were tested on two different year classes of delta smelt, as well as at varying drug doses and temperatures.

For each assay a bath treatment was prepared by measuring 10L of LSNFH water into an 18L bucket, and adding the treatment dose. The bath treatment was conducted on 30 fish per treatment for 1 hr. Two buckets served as a control group, with 30 fish each, and these fish received a static bath of LSNFH water only. Fish were checked every 15 minutes during treatment for adverse reactions to the chemical compounds (signs of distress or mortality). After the bath treatment, all treated fish were placed in circular holding tanks. One control group was placed in a circular holding tank and allowed to swim freely, while the other control group was placed inside a live cage for holding. The live cages were constructed of 6 inch PVC pipe with 3" holes covered by quarter inch mesh for water exchange. All fish were monitored for mortality for 72 hours.



Salt bath treatment (left) and control group (right)

Mortality data showed that there was no difference between the three treatments tested. The highest mortality seen was in the treatment of one year old delta smelt treated with Rid-Ich+ at a low temperature. There was no mortality recorded in any of the control groups. A summary of mortality data is shown in Table 1.

Treatment	Dose	Year Class	Average Water Temperature °C	Percent Mortality
Salt	1%	1 year old	9.1	0.0%
		2 year old		3.3%
Salt	2%	2 year old		3.3%
		1 year old		6.7%
Chloramine T	15mg/L	2 year old		0.0%
		2 year old		3.2%
Rid-Ich+	1.5ml/L	1 year old		20.0%
		2 year old		0.0%
Rid-Ich+	1.5ml/L	1 year old	12	3.3%

Table 1 - Summary of Mortality Data

All data was analyzed using Fisher's Exact Test (2 tailed) with an alpha level of .05. There was no statistical difference between treating the same year class with the varying dosage of salt ($p = 1.5$) or treating different year classes with the same dose of salt ($p = 1.0$). The results were the same for Chloramine T ($p = 1.0$, $p = 0.49$) respectively. There was no statistical difference between treating the same year class with the same dose of Rid-Ich+ at varying temperatures ($p = 0.10$).

There was a significant difference when treating different year classes with the same dose of Rid-Ich+ at the same temperature ($p = 0.02$). In January, the Rid-Ich+ treatment on the one year old fish had the highest mortality, at 20%, compared to ~3-6% for other treatment doses, temperature and size classes tested. Therefore, this assay was repeated in June when water temperature had increased by three degrees. The mortality at the higher temperature was 3%. This difference was not statistically significant ($p=0.10$) between temperature regimes tested.

With the exception of 1% salt, 2 year old fish had lower mortality than 1 year old fish treated with Chloramine T (15mg/L) or Rid-Ich+ (1.5ml/L). The Rid-Ich 1.5mg/L treatment in 1 year old fish (9.1C) incurred 20% mortality compared to no mortality at the same temperature.



Fisheries Biologist working with Delta Smelt

Handling was kept to a minimum to reduce stress and crowding was taken into consideration as well; the treatment buckets had a calculated density index of .16 (Piper, 1982). Thirty fish were handled to collect length and weight data, however these fish were not used in the bioassay treatments, as the additional handling would likely produce false results attributed to excessive handling rather than the chemotherapeutants being tested.

Rearing practices at both FCCL and LSNFH have found that delta smelt experience less stress and mortality when maintained in dark environments, and allowed to swim freely versus being held in live cages. For example, black colored or camouflaged holding tanks or

buckets are preferred over white tanks. A control group placed in live cage for 72 hours experienced an average mortality of 72% compared to the same group of fish allowed full use of 4 foot circulars. However, for the purpose of this bioassay, white buckets were used for all treatments so fish could be observed and monitored over the one hour treatment time. Fish behavior did not indicate that the fish were overly stressed while being treated, and the mortality observed in side by side comparisons can most likely be attributed to a biological response to the chemotherapeutants. The challenges associated with simple handling, and holding of this species in experimental units reiterates the fact that delta smelt are can be a difficult species to study.

The purpose of this study was to investigate potential treatments for ectoparasite or bacterial infections that the California-Nevada Fish Health Center could utilize to support culture operations for the LSNFH delta smelt program. There was no evidence that delta smelt incur high mortality from chemotherapeutic toxicity at the doses tested. Temperature and age of fish was not a factor with the exception of the 20% mortality observed in 1 year old fish treated with Rid-Ich. Young fish may be more susceptible to this compound, which is a combination of malachite and formalin, however this treatment regime should be repeated to ensure the increased mortality was not due to handling of this particular group of fish.

Monitoring Mycobacterium Infection in Production and Broodstock Groups

Mycobacteriosis is a disease caused by certain bacterial species within the genus *Mycobacterium* (Francis-Floyd, 1999). It is likely that all fish species are exposed to *Mycobacterium* because of the ubiquitous distribution in aquatic environments (Antonio D.B, 2000; Whipps, 2007).

The disease is of concern in fish because it is a chronic in nature, can cause significant mortality within affected populations, and few compounds are effective in treating epizootics once they occur. Outbreaks in wild fish have documented loss of fitness and increased predation for infected fish (Poort, 2006). Cultured delta smelt stressed from handling, spawning, or increased water temperatures have a higher prevalence and severity of mycobacterium infections (Antonio D.F, 2000). It is also suggested that mycobacterium can be transferred horizontally via shedding of mycobacterial cells, although ingestion is probably the main route of infection (Antonio D.B, 2000). Vertical transmission of



Delta smelt muscle/skin lesion above the lateral line.

mycobacterium has been studied in other fish species (Ross, 1962) and was found to occur in aquarium fish (Chinabut, 1994); consequently it is suspected to occur in delta smelt as well (Antonio D.B, 2000; Francis-Floyd, 1999). Public health concerns also exist because individuals, usually immunocompromised or with pre-existing cuts or wounds on the hands, can contract a skin infection (fish tank granulomas) when working with water containing the bacteria, or brood fish that have active infections.

Signs of mycobacteriosis in fish are variable (Francis-Floyd, 1999), but generally include bacterial granulomas in various tissues. Affected fish can appear lethargic, emaciated, and commonly develop nodular lesions in the mouth/jaw areas, or the musculature under the skin. Internally, granulomas may develop in the kidney, liver, or spleen (Francis-Floyd, 1999; Mycobacteriosis).

Presumptive diagnosis of mycobacteria can be based on the presence of granulomas containing acid fast bacteria (Francis-Floyd, 1999), however traditional diagnosis is by bacterial culture and differential biochemical tests (Ucko, 2002). Diagnosis by culture is not always conclusive because the bacteria are very slow growing and can be prone to competition by masking bacteria during their long generation time (Plikaytis, 1992). Therefore molecular tools like QPCR can be useful in diagnosis of both low level and early infections.

Mycobacterium was detected previously at the FCCL in Byron during a diagnostic exam in 2005. At that time 2/3 fish tested presumptively positive for *Mycobacterium*, based on

the history provided and the presence of acid fast bacteria (Ca-Nv FHC unpublished data). In 2007, LSNFH experienced chronic mortality in spawned females that were being re-conditioned for a second spawn. Mortality had been chronic for over 3 months in the group of post spawned females. Upon gross clinical examination, approximately 73% of the fish had developed lesions along the lateral line musculature and in the mouth and lower jaw. Mycobacteria were suspected, due to the clinical presentation and disease history of the wild source population incorporated in to the FCCL broodstock. Of the clinical fish tested, 9/27 (33%) tested positive for acid fast bacteria, a presumptive identification. Clinical fish liver and spleen tissues were cultured and found to be 67 % positive for mycobacteria. Culture identification was verified by a second laboratory (USGS-Western Fisheries Research Center). Genotyping was performed on 7 isolates submitted to Chris Whipps (Whipps, 2007) to further identify the specific mycobacteria species that isolated from clinical fish. Three species of *Mycobacterium* identified were *M. marium*, *M. chelonae* and the closely related sub-species *M salmoniphilum*.

Mortalities of delta smelt at LSNFH are screened for mycobacteria by QPCR and in 2010, four cases were tested (Table 2). Case 10-001 was the fish health inspection prior to fish transfer from FCCL to LSNFH. Case 10-025 tested delta smelt adult mortality post spawning, case 10-072 tested a subset of mortalities, and case 10-103 was a diagnostic case to resolve mortality in larval production units. In each case, liver tissue was collected and extracted, and total DNA was submitted to the Diagnostics Core Facility at the University of California, Davis for QPCR testing.

Case Type	Case Number	Assay Method	Number of Samples	Total Number of Fish	Number Positive	Percent Positive for Mycobacterium
Inspection	10-001	QPCR	10	50	30/50	60%
Diagnostic	10-025	QPCR	30	30	23/30	77%
Diagnostic	10-072	QPCR	30	30	17/30	57%
Diagnostic	10-103	QPCR	4	12	12/12	100%

Table 2 - Summary of *Mycobacterium* testing (Testing was not focused on fish with signs of disease, but instead was conducted randomly. One exception is case 10-025, which did have one clinical mortality. Both liver and muscle lesions from this fish were tested and both tissues are included in the total number of fish).

Biosecurity Measures

A Hazard Analysis and Critical Control Point (HACCP) plan for delta smelt transport is in place to transport delta smelt from the FCCL facility to LSNFH (Service U. F., 2006). This protocol is designed to prevent potential hazards and unwanted targets from entering the facility. These hazards include pathogens as well as plants, invertebrates, or biological contaminants.

Following the outbreak of mycobacteria at LSNFH in 2007, additional biosecurity measures were implemented to prevent the spread of this bacterium at this facility (Service U. F., Oct 2007). Housing fish in isolated small mixed family groups, controlled cooler water temperatures for rearing and spawning operations, and UV disinfection of both incoming water and hatchery effluent were the key steps. Additional measures were implemented to prevent direct transmission between fish, and to minimize the exposure of hatchery workers who handle fish during routine husbandry operations and spawning. These measures included dedicated equipment for each isolation group, use of disinfecting footbaths and hand sanitizers, and use of prophylactic oxytetracycline water baths when handling adult fish for spawning. Equipment used for rotifer and artemia food production is also disinfected between uses.

Fish health inspections and testing subsets of suspicious or post-spawn mortality for mycobacterium will be continued as necessary to monitor these bacteria in the production and refugial populations reared at LSNFH. Despite relatively high prevalence of mycobacteria in the sub-adult transfers (60%) and post spawning adults (77%), we have not experienced disease problems associated with mycobacterial infections in the past 3 years of production. Best culture practices that minimize handling and stress, along with biosecurity measures aimed at reducing direct transmission, appear to be effective in managing a potential fish pathogen that is endemic to this species.

Summary

The California-Nevada Fish Health Center provides fish health services and culture support to LSNFH. Monitoring work conducted in FY2010 provides information that can be used in future management of this important resource. The chemotherapeutant bioassays indicate 1 and 2 year old delta smelt can tolerate commonly used parasiticides and water disinfection compounds at temperatures ranging from 9-12°C. When treating with salt or Chloramine-T, no difference in mortality was seen if the dosage, temperature, or age class was varied. The only significant difference in mortality was seen when treating 1 year old fish with Rid-Ich but this may have been due to handling effects and should be repeated to verify this result. In general, 2 year old fish had lower mortality in two of the treatments when compared to 1 year old fish. The bioassay suggests that if a disease outbreak should occur, the Ca-Nv FHC could treat delta smelt with these chemotherapeutants without high mortality from the chemotherapeutants themselves.

Mycobacteria testing will be continued in 2011 on a sub-set of production and adult mortalities from LSNFH. The samples will continue to be submitted to UCD for QPCR testing, however the Ca-Nv FHC will consider designing an in-house QPCR assay that is more specific to the 4 species of mycobacteria detected in delta smelt to date.

Difficulties remain in terms of maintaining strict biosecurity for the facility and program in two key areas. The transfer of live sub-adult fish each fall, to complement the refugial population to the FCCL, poses a significant fish health risk in terms of the potential introduction of fish pathogens to the LSNFH. However, it would be difficult to maintain a genetic refugial population in any other manner than the current practice of transferring a representative composition of delta smelt to LSNFH as sub-adults.

The logistics of matching the genetic composition and proportions of mixed family groups held at FCCL using disinfected egg transfers, while safer than live fish transfers, cannot be overcome with current funding and facility design. The second vulnerability is the use of aquatic snails, from uncontrolled sources, to control algal growth in larval tanks. Commercial specific-pathogen-free (SPF) snails are not currently available and use of snails from a local creek poses a significant fish health risk for introduction of ectoparasites, helminthes, bacteria or viruses that may be shared between snails and fish. General disinfection protocols and chemical treatment efficacy for snail culture will be evaluated in FY2011 to further reduce this remaining risk.

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Appendix I

Sample Summary – 2009 Delta Smelt

Facility: Byron Fish Culture Facility, Bryon, CA
Stock: 2009 Delta Smelt – Fish Health Exam for transfer of sub-adults to LSNFH

Summary

Sub-adult delta smelt (*Hypomesus transpacificus*) were submitted for fish health inspection on October 8, 2009 (Lab Case 10-001). Live fish were euthanized, necropsy was performed, and tissues processed for specific pathogen assays including screening for Mycobacterial infection. No reportable pathogens were detected. *Mycobacterium* was detected by QPCR screening.

Table 1. Summary of Tests and Prevalence of Infection (POI) by assay and pathogen type.

Assay (Method)	# Samples (pool size)	Total Fish	Pathogens Screened	# Positive (% positive)
Virology (Tissue Culture – EPC/CHSE cell lines)	30 (5)	150	IHN, VHSV, IPNV, OMV ¹	0/30
Culturable Bacteria (BHIA culture)	30 (1)	30	<i>Aeromonas salmonicida</i> ¹ <i>Yersinia ruckeri</i> ¹	0/30 0/30
QPCR	10(3)	30	<i>Mycobacteria</i> spp. ³	6/10 (60%)

Notes: ¹ Pathogens listed in the USFWS Fish Health Policy (713 FW 1-5) as reportable pathogens associated with salmonid disease.

² Additional testing for presence of *Mycobacteria* species was done by culture on selective media (Middlebrook) and Acid-Fast (AF) Stain of bacterial isolates.

³ Extracted DNA from tissue samples were sent to UC Davis, Lucy Whittier Molecular and Diagnostic Laboratory for *Mycobacteria* spp. screening by QPCR.

Laboratory Methods

Virology (IHN, IPNV, VHSV, OMV): Inoculation of 5-pool viscera tissues onto EPC and CHSE-214 cell lines. Observed for viral cytopathic effects (CPE) over an 18 day incubation period (18°C). Blind passage of negative or suspect samples at 21d, observed for an additional 7d (18°C).

Culturable Bacteria: *Aeromonas salmonicida* (AS) and *Yersinia ruckeri* (YR) assayed by direct culture of individual kidney tissue onto appropriate growth media (BHIA) and subsequent biochemical testing (OF, TSI) of all presumptive bacterial isolates.

Mycobacterium Screening: Extracted DNA from tissue samples were sent to UC Davis, Lucy Whittier Molecular and Diagnostic Laboratory for *Mycobacteria* spp. screening by QPCR.